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Latex yield and quality during storage of guayule (*Parthenium argentatum* Gray) homogenates

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Abstract

Extraction and purification of latex from guayule (*Parthenium argentatum* Gray) require that harvested shrub first be homogenized in an alkaline aqueous buffer. The stability of the latex was investigated in a variety of homogenates and under different storage conditions. Neither the length of post-harvest storage (up to 5 weeks) nor branch diameter affected the concentration of the latex in homogenates. Latex concentration was not affected by the length of grinding time used to make the homogenate. However, latex concentration declined in acidic pH, and the initial latex concentration in homogenate prepared from defoliated shrub was below 4 mg/ml. This decline was less apparent in homogenates made from leafy shrub, suggesting a protective effect derived from the leaves. Storage at 4 °C prevented latex loss under all treatments.

The quality of the rubber polymers in the latex fraction was investigated using size exclusion chromatography/multiangle laser light scattering detection. Polymer molecular weight and molecular radius declined in parallel, but both declined faster in the dilute homogenate generated from the second grind of the guayule bagasse than in the more concentrated homogenate from once-ground shrub. Degradation was greatly slowed in all treatments when the homogenates were stored refrigerated. Polydispersity values were low in all treatments, only slightly increasing over time, with the exception of homogenate generated by the second grind of shrub in the presence of leaves and stored at 24 °C. The relatively rapid polymer degradation of the latex fraction in this homogenate led to an increase in polydispersity followed by a decrease as the latex fraction was degraded to below detection levels.

We conclude that guayule homogenate provides a stable environment for latex yield and quality, even at room temperature, for at least 13–16 weeks provided that the pH is basic and the concentration of rubber particles is at least 4 mg/ml. This is in contrast to the extractable latex content of harvested branches, which is prone to rapid coagulation and degradation in situ unless the branches are stored hydrated and refrigerated.

Keywords: Guayule; Homogenate; Hypoallergenic latex; Storage

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1. Introduction

Parthenium argentatum (Gray), commonly known as guayule, is a perennial, woody shrub native to the Chihuahuan desert of the United States and Mexico (Whitworth and Whitehead, 1991), currently under development as a source of high quality, low protein, hypoallergenic latex (Cornish, 1996, 1998). This latex provides a safe alternative source of natural rubber latex for people suffering from life-threatening Type I latex allergy (Siler and Cornish, 1994; Carey et al., 1995; Siler et al., 1996) and is suitable for the manufacture of high-quality medical products (Cornish and Lytle, 1999; Cornish et al., 2001). Factors affecting the extractability and stability of latex rubber in harvested guayule shrub are under investigation in order to maximize latex yields, and optimize post-harvest practices (Cornish et al., 1999, 2000). Initial studies demonstrated that the extractable latex levels in harvested branches of different size are stable for at least 2 weeks when sealed in plastic to prevent dehydration and stored at 4 °C. However, even slight dehydration reduced the extractable latex content, and large losses were observed at warmer temperatures (Cornish et al., 2000).

Guayule shrub must be homogenized to extract the latex (Cornish et al., 1999), but until this study, no information was available on the stability of the latex fraction in the homogenate. Therefore, we have investigated the stability and quality of the latex fraction in homogenates prepared in a variety of ways and stored with and without refrigeration.

2. Materials and methods

2.1. Plant material

Preliminary experiments indicated that homogenate from different guayule lines would behave similarly during storage. In this report, branches were harvested from mature guayule shrub (≥3 years old), line 11591, field-grown at the U.S. Water Conservation Laboratory, Phoenix and at the University of Arizona Experimental Station, Maricopa in Arizona. Branches were dipped into 1% aqueous ascorbic acid, as an antioxidant, sealed in plastic, and shipped overnight, on ice, to Albany, California.

Some harvested branches were sorted into three branch diameters, which approximated the relative age of the different tissues: small: <0.5 cm, medium: 0.5 to <1.0 cm, large: >1.0 cm diameter. The smallest branches contain very little woody material and represent the current year's growth. The medium branches are over a year old, contain a more developed woody core, and have experienced one winter season. The largest branches are at least two years of age, contain a fully developed woody core, and have experienced at least two winters. All sealed shrub samples were stored at 4 °C until processed.

2.2. Homogenate

Filtered homogenates were prepared according to the Waring blender method previously described (Cornish et al., 1999) at room temperature, using 2 × 60 s grinds, a total of 1:4 (w:v) of shrub to aqueous extraction buffer (0.2% ammonia, as NH_4OH, 0.1% Na_2SO_3, pH 10), and a 1-mm filter. Depending upon the experiment, the filtered homogenates from the different grinds were stored separately or after they were pooled. In some experiments, homogenates were made separately from different branches sizes, whereas in others, the branch sizes were pooled before homogenization. Except where otherwise stated, all homogenates were prepared from defoliated branches. Homogenates were stored in 100 ml sealed glass bottles at room temperature (24 °C) or at 4 °C until quantification of their latex concentrations. Unless noted otherwise, the pH of the homogenates was maintained at pH 10 using NH_4OH, by first readjusting to pH 10 immediately after the homogenate was prepared, again during the next day, and then as indicated by weekly pH checks.

2.3. Latex quantification

Latex concentration in all homogenates was quantified using the 1 ml quantification method previously described (Cornish et al., 1999), where 3 × 1 ml aliquots of homogenate are centrifuged to float the latex fraction, which is then coagulated with glacial acetic acid, harvested, rinsed, dried at 37 °C and weighed.

2.4. SEC-MALLS rubber polymer analysis

Coagulated, dried rubber samples generated by the latex quantification method and dried overnight at 37 °C, were stored under N_2 at −80 °C in 1.5-ml

microfuge tubes until analysis. Samples were weighed out into 8-ml glass vials with Teflon-lined caps. Tetrahydrofuran (THF) (prefiltered through a 0.2-μm vacuum filter) was used to dissolve the rubber and generate a concentration between 0.3 and 2.5 mg/ml and left overnight. Some rubber coagula were too small to be analyzed individually, and in these cases, the three replicates were pooled before analysis. Samples held more than one day before analysis were stored under N_2. The dissolved samples were shaken vigorously and 1-ml aliquots were filtered through 0.45-μm syringe filters composed of PTFE with GMF 25 mm disposable GD/X filters (Millipore, Burlington, MA) into autosampler vials with Silicone/Teflon-lined septa. Each sample solution (100 μl) was analyzed using a HPLC/MALLS system consisting of a HP 1100 series autosampler, a pump, a HP1047 refractive index detector (Agilent Technologies, Palo Alto, CA), a multiple angle laser light scattering detector containing 18 light scattering detectors with a 632.8 nm wavelength laser (Dawn DSP Laser Photometer, Wyatt Technologies, Santa Barbara, CA), a Phenogel 5 μm Linear/Mixed Guard Column (Phenomenex, Torrence, CA) and a PLgel 10 μm Mixed-B-size exclusion column (Agilent Technologies, Palo Alto, CA) maintained at 35 °C. Latex rubber polymers were eluted within 17 min by THF (1.0 ml/min). Astra software (Wyatt Technologies, Santa Barbara, CA) was used to calculate molecular weight (weight-averaged, M_w) and root mean square (r.m.s.) radius moments for each peak, as well as polydispersity (M_w/M_n), a measure of diversity in molecular weight. A general description of the use of light scattering for molecular characterization, calibration of the light scattering detector and equations for calculating the various parameters are provided in a review by Wyatt (1993).

2.5. Statistical treatment

Data were treated by regression analysis with plots dictating the model form, because little is known about what shape should fit. In many instances, non-linear curve fitting was needed with asymptotic or logistic models being the chosen models. When no discernible change was apparent, a linear regression was applied, and when no clear pattern was evident, polynomial fitting was performed up to the third power. Deviations from the model were used to graph confidence intervals on the resultant prediction lines. Regressions were performed with regression and non-linear procedures available in the SAS software (SAS Institute Inc., 1999; Freund and Littell, 2000).

3. Results

3.1. Latex concentration

Latex concentration in homogenates made from grinding branches for 60 s (first grind, circular symbols) was mostly stable throughout a 24-week storage experiment (Fig. 1A, C–E), as shown by the non-significant slope of the regression line fitted to weeks. However, homogenate made from the largest stems that had been stored post-harvest for 5 weeks at 4 °C (Fig. 1F) had a significant decrease ($P = 0.004$), and homogenate made from medium-sized stems stored for 2 weeks (Fig. 1B) showed a slight decline in latex content ($P = 0.03$). Thus, the latex content in most first-grind homogenates, independent of original branch size, showed little decline over 24 weeks whether or not the shrub had been stored for 2 (Fig. 1A–C) or 5 (Fig. 1D–F) weeks prior to homogenization. In contrast, the latex fraction was much less stable in homogenates made from grinding the bagasse from the first grind for another 60 s (second grind). The latex concentration appeared to remain stable for a time, followed by a period of decline, sometimes quite rapid, then another period of little change. Because of this S-shape, data from grind 2 were fitted to a logistic model (Fig. 1A–F). Similar patterns of decline were observed regardless of the length of time that the branches had been stored prior to homogenate preparation, or their age, although the rapidity of decline varied.

Storage experiments were performed to investigate the cause of the decline in latex concentration seen in the homogenate from the twice-ground branch samples (Fig. 1). In these experiments, homogenates were made from a pooled sample of guayule branches reflecting all three branch diameters used in the earlier experiments and ground as specified for each particular experiment. These experiments with homogenates included tracking latex concentration over time in relation to pH, grinding for different times before storage, grinding with and without leaf tissue, and different storage temperatures.

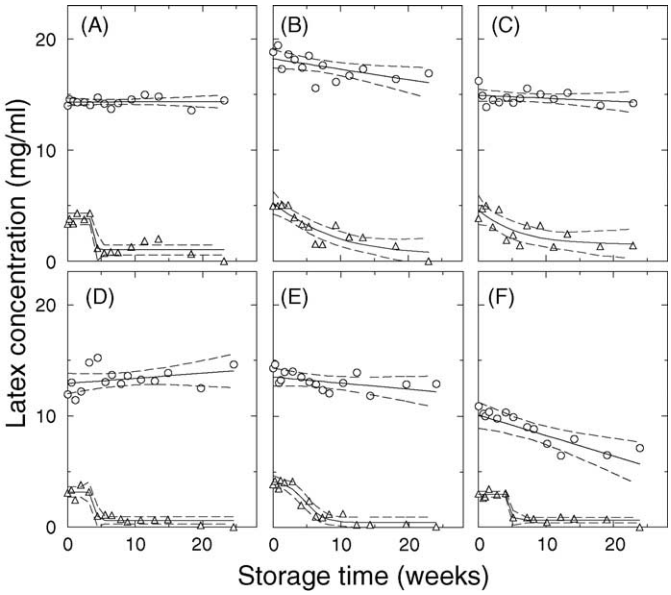

Fig. 1. Latex stability in stored homogenate made from guayule branches of three different diameters: <0.5 cm (A, D); 0.5–1.0 cm (B, E); >1.0 cm (C, F). Branches were stored in sealed plastic bags at 4 °C for 2 (A–C) or 5 weeks (D–F), then ground for 1 min and filtered before storage at room temperature (24 °C) (first grind, ◯). All homogenates were adjusted to pH 10 before being stored, but were not adjusted again. Each value is the mean of three latex quantifications. Data from grind one were fitted to a regression model, whereas data from grind 2 were fitted to a logistic model. High and low confidence limits (95%) are indicated by the dashed lines.

Latex concentration decreased over time and the reduction was greater when pH was uncontrolled (Fig. 2). When the pH was maintained at pH 10, latex concentration was only reduced 1.3 mg/ml in 36 weeks, a 24% reduction. The rate of decline in the uncontrolled latex increased rapidly after 22 weeks, although the pH had dropped to below pH 7 after only the first week of storage, possibly due to growth of lactic acid bacteria. The major latex decline after 22 weeks was much later than the instability observed earlier after only 3 weeks (cf. Figs. 1 and 2). However, an early smaller decline in latex concentration was also observed in this experiment.

Also, we investigated the possibility that 120 s of grinding compared with only 60 s was the cause of the observed decline in latex content (Fig. 1) perhaps by damaging the rubber particle membrane (Cornish et al., 1999, 2000) and facilitating coagulation and microbial attack, or by releasing more of guayule's own degradative enzymes. Results in Fig. 3 show an asymptotic model fit to the data, indicating that the latex content stabilized after an initial decline. Instead of causing the observed decline in latex content, grinding shrub

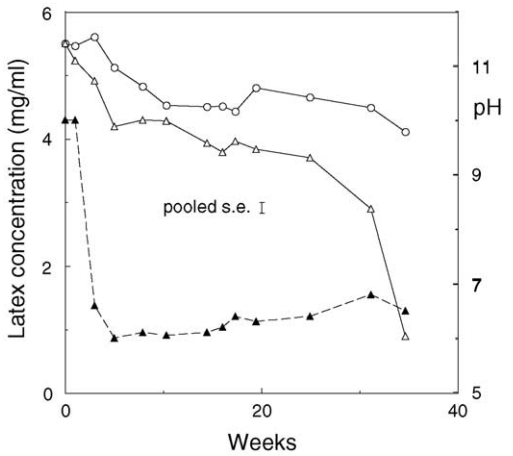

Fig. 2. Latex concentration during storage of guayule homogenate either maintained at pH 10 through regular adjustment with NH_4OH (◯), or unregulated (Δ), in which pH changed as shown (▲). Homogenates were stored at room temperature (24 °C). Each value is the mean of three latex quantifications. The pooled standard error ($\sqrt{\sum}(S.E.)^2/n$) of the mean applies to all the latex quantification data.

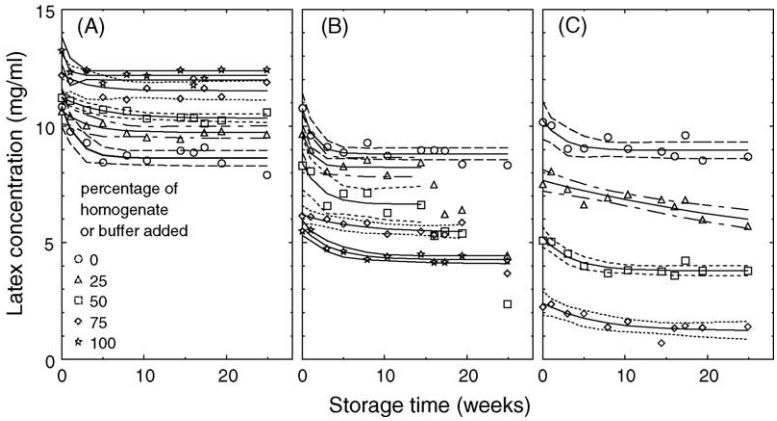

Fig. 3. Effect of initial latex concentration and grinding time on latex stability. The homogenates were prepared by mixing homogenates prepared by grinding guayule shrub for 60 s with either (A) homogenate ground for 120 s, (B) homogenate generated by grinding the bagasse remaining after the homogenate from a 60 s grind was filtered off, for an additional 60 s, or (C) with aqueous 0.2% NH_4OH and 0.1% Na_2SO_3 homogenate (grinding solution). Homogenates were stored at room temperature (24 °C) and maintained at pH 10. Each value is the mean of three latex quantifications. The data were fitted to an asymptotic model, and 95% confidence limits are indicated by the fine lines.

for 120 s actually increased the amount of latex recovered; the initial latex concentration increased in direct proportion to the amount of added 120 s ground homogenate added (Fig. 3A), reflecting a more complete extraction of the latex from the branches. All levels stabilized after the initial decrease with the level of the final asymptope directly proportional to the amount of homogenate added. Non-overlapping confidence intervals verify the significant difference among asymptopes. In contrast, homogenate mixtures containing homogenate obtained by regrinding the bagasse obtained after an initial 60 s grind, for another 60 s (the same conditions as "second grind" in Fig. 1), had initial amounts and asymptotic levels running in the opposite direction (Fig. 3B) as the latex concentration in the initial 60 s homogenate was diluted with the homogenate from the second 60 s grind. The asymptotic model was fit and non-overlapping confidence intervals indicate significant differences among proportions of homogenate added in the expected order. For intermediate proportions (25, 50 and 75%), the latex concentration took a rapid decline after 15–20 weeks that could not be modeled. This loss of latex after 15–20 weeks did not occur when the homogenate was diluted with buffer, possibly due to a higher level of the antioxidant Na_2SO_3 in the mixture (Fig. 3C). It should be noted that, in this experiment, the 120 s ground homogenate has a higher latex content than the 60 s ground homogenate (Fig. 3A), in apparent contrast to the data in Figs. 1 and 2. This is because in Fig. 3A, a subfraction of the 60 s ground homogenate was ground for an additional 60 s with no additional buffer. The data in Figs. 1 and 2 reflect the latex concentrations in a homogenate filtered after 60 s and another made by regrinding the bagasse in additional buffer. In these cases, of course, the second grind homogenate had a much lower latex concentration, because most of the latex had already been removed from the branches during the first grind.

The effect of temperature and of leaves during homogenization on latex concentration during long-term storage also was investigated. Leaves on guayule shrub present a large additional mass of material and necessitated considerably more grinding buffer to make the homogenate. Because the leaves do not contain rubber, this led inevitably to a lower latex concentration in the homogenate (cf. Fig. 4A and B). Linear regressions described the data quite well. Substantial declines in latex concentration were observed in the second grind homogenate stored at 24 °C, as shown by significant slopes in the leafy (Fig. 4A) and defoliated (Fig. 4B) shrub homogenates, $P = 0.0009$ and 0.0001, respectively. A significant decline in latex content also was seen in defoliated shrub homogenate from grind 1 stored at 24 °C ($P = 0.0015$). However, no latex losses were observed in either

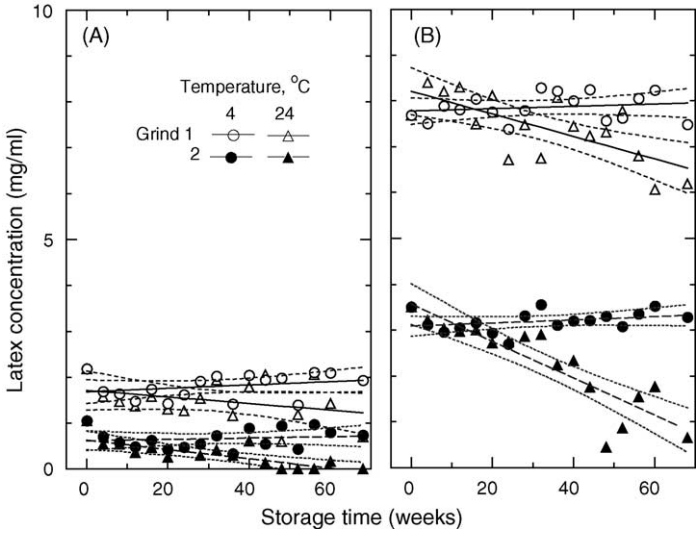

Fig. 4. The stability of latex in guayule homogenate produced by two consecutive 60 s grinds of (A) shrub with leaves attached and (B) defoliated shrub. Homogenates were subdivided and then stored at room temperature (24 °C) or at 4 °C. Each value is the mean of three latex quantifications. Linear regression was used to describe the data, and 95% confidence limits are indicated by the fine lines.

homogenate stored at 4 °C over the 66 weeks of the experiment.

3.2. Latex quality

Rubber molecular weight is closely correlated to rubber quality (Swanson et al., 1979), and so, this parameter and other molecular characteristics were used in this investigation to monitor the stability of latex quality during the homogenate storage experiment described in Fig. 4. Initial molecular weights were above 1,000,000 g/mol, but declined in all treatments during storage (Fig. 5). A logistic model was used to describe the data, except where week 0 or 6 data were missing. These cases did not allow for fitting the initial time before the start of the decline and an asymptotic model had to be used. At 4 °C, molecular weights decreased to approximately 500,000 g/mol after 40 weeks. The rate of degradation was consistently greater at 24 °C than at 4 °C, reaching lows at or below 100,000 g/mol, and with confidence limits not overlapping those at 4 °C. The level reached in the more dilute second grind homogenate was generally lower than in homogenate from the first grind, although the difference is only clearly significant for homogenate containing leaves and stored at 4 °C; the time of decrease in earlier for data from homogenate stored at 24 °C. At 24 °C, molecular weights decreased to 800,000 g/mol in 16–17 weeks in the homogenate generated by grinding guayule shrub for 60 s, but in only 4 and 8 weeks in the more dilute homogenate from the second grind of shrub with leaves or defoliated, respectively (Table 1). In contrast, at 4 °C, it took at least 23 weeks for rubber molecular weight in even the least stable latex to decline to 800,000 g/mol. In all treatments, the rate of decline was not constant and slowed over time. Latex samples from homogenate from the second grind of leafy shrub and stored at 24 °C could not be analyzed beyond 37 weeks because not enough latex remained for analysis.

As expected, r.m.s. radii of the rubber molecules decreased (Fig. 6) as the molecular weight decreased (Fig. 5), although the relative decrease in radius was less severe than that of the molecular weight. As done for the molecular weight, a logistic model was used to describe the data, except for homogenate made from leafy shrub by grind 2 and stored at 24 °C where an asymptotic model had to be used. As with molecular weight, decreases appeared to be greater at 24 °C than at 4 °C. However, only clearly significant differences occurred with homogenates made from defoliated shrubs where rubber molecular radii in homogenates from

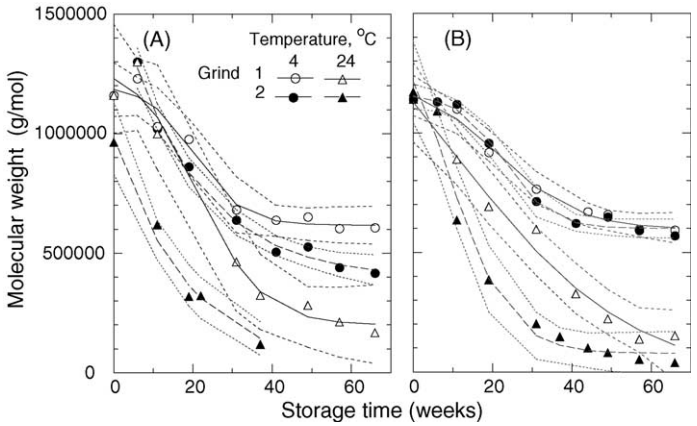

Fig. 5. The molecular weight of the rubber in the extractable latex fraction of homogenates produced by two consecutive 60 s grinds of (A) shrub with leaves attached and (B) defoliated shrub. Homogenates were subdivided and stored at 24 °C (room temperature) and at 4 °C. Logistical or asymptotic models were used, respectively, to describe data, with or without an initial shoulder before the start of the decline, and 95% confidence limits are indicated by the fine lines.

both grinds decreased more when stored at 24 °C than at 4 °C, and grind 2 declined earlier than grind 1 under the same storage conditions.

The polydispersity (M_w/M_n) of the rubber molecules in stored homogenates ranged from 1.1 to 2.6 (Fig. 7). As a reference, a polydispersity value of 1 indicates a polymer of uniform molecular weight, and rubber latex extracted from guayule branches stored at 40 °C can rise to a polydispersity index of nearly 4 (Cornish et al., 2002a, 2002b). The polydispersity values for the latex rubber in stored homogenates were mainly all quite low, although generally a little higher at 24 °C than in homogenate stored at 4 °C. The largest changes in polydispersity at 24 °C were observed in the dilute second grind latex containing leaf material (Fig. 7A) that degraded most rapidly (Figs. 4A and 5A).

The radii/molecular weight ratio, a measure of molecular conformation (Fig. 8), indicated that the guayule latex rubber molecule is a random coil (ratio range of 0.5–0.6), except in severely degraded rubber at 24 °C, where the conformation became more compact and spherical (ratios down to 0.14). The random coil conformation was unaltered during refrigerated storage.

Polynomial regression was used to model these data since there was no evidence of a non-linear pattern as seen for some of the previous variables. For

Table 1
The storage time required to degrade, to particular molecular weights, the rubber in the extractable latex fraction of ammoniated homogenates. Storage times were calculated using the regression models in Figure 5.

Rubber molecular weight (g/mol)	Storage temperature (°C)	Homogenate storage (weeks)			
		Shrub with leaf		Defoliated shrub	
		Grind 1	Grind 2	Grind 1	Grind 2
1,000,000	4	17	13	15	17
	24	14	0	6	5
800,000	4	25	21	29	26
	24	20	4	16	10
500,000	4	>66	46	>66	>66
	24	29	13	31	16

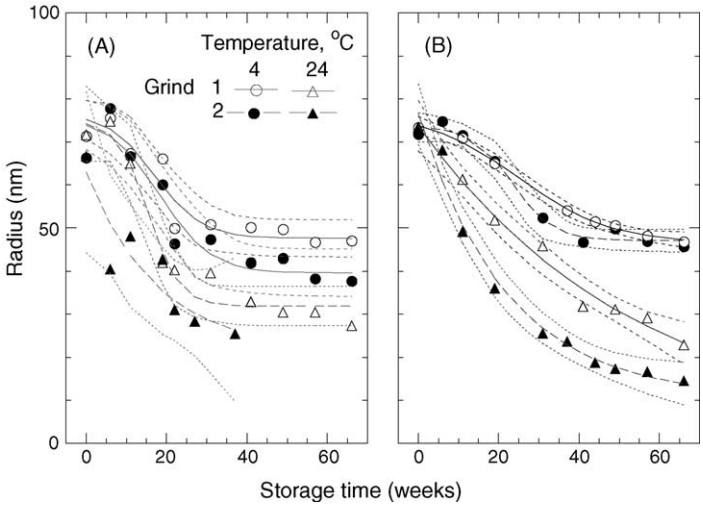

Fig. 6. The molecular radius of the rubber in the extractable latex fraction of homogenates produced by two consecutive 60 s grinds of (A) shrub with leaves attached and (B) defoliated shrub. Homogenates were subdivided and stored at 24 °C (room temperature) and at 4 °C. Logistical or asymptotic models were used, respectively, to describe data, with or without an initial shoulder before the start of the decline, and 95% confidence limits are indicated by the fine lines.

homogenates stored at 4 °C, there is no clear difference between grinds 1 and 2, although grind 2 has a significant regression (linear in homogenate from leafy shrub, and quadratic in homogenate from defoliated shrub). Large differences were observed in homogenates stored at 24 °C, all of which show a significant decline in ratio, with the decrease being greatest for the grind 2 data. The difference between the grinds was most pronounced for leafy shrub.

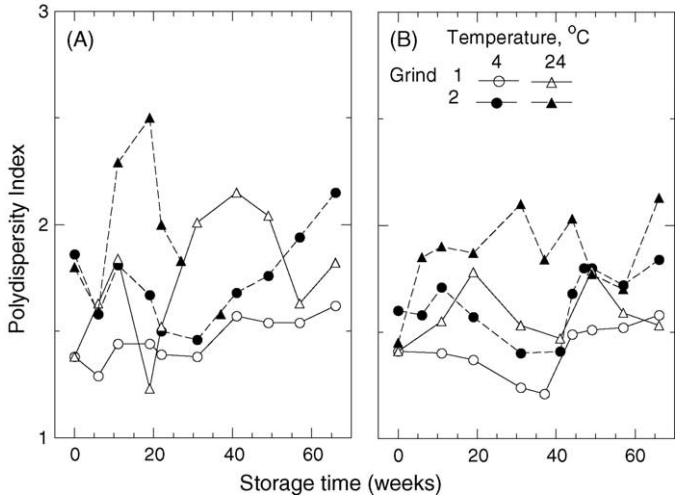

Fig. 7. The polydispersity index of the rubber in the extractable latex fraction of homogenates produced by two consecutive 60 s grinds of (A) shrub with leaves attached and (B) defoliated shrub. A value of 1 would be generated by a polymer of uniform molecular weight (a monodispersed sample). Homogenates were subdivided and stored at room temperature (24 °C) and at 4 °C.

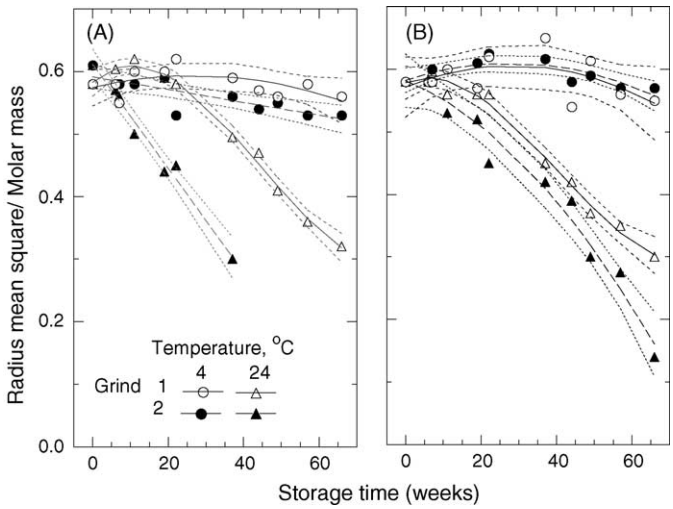

Fig. 8. Molecular conformation plot of the rubber in the extractable latex fraction of homogenates produced by two consecutive 60 s grinds of (A) shrub with leaves attached and (B) defoliated shrub. A value of 0.33 is generated by a spherical polymer, 1 by a rod, whereas 0.5–0.6 reflects a random coil, the form taken by fresh guayule rubber. Polynomial regression was used to model the data, and 95% confidence limits are indicated by the fine lines.

4. Discussion

4.1. Latex concentration

It is clear that ammoniated guayule homogenate provides a much more stable environment for latex than harvested shrub (Cornish et al., 2000), and latex levels can be maintained almost indefinitely under refrigeration. Ammoniation raises the pH to 10, a level that severely inhibits microbial growth and enzyme activity. Even in the least stable homogenate (Fig. 1), latex levels did not begin to decline until after 3 weeks at 24 °C. At this temperature, longer storage times (at least 8 weeks) may be achieved without latex degradation provided that basic pH is maintained and that the initial latex concentration is at least 4 mg/ml. The initial latex concentration of the homogenate (Figs. 1 and 4B) did appear to be a critical factor because only the most dilute latex concentrations lost a substantial portion of their latex. Also, when homogenate was diluted threefold with buffer, to a latex concentration of 2.25 mg/ml, the latex was lost more rapidly from this dilute sample (Fig. 3C). The underlying causes for latex loss from dilute concentrations are unclear but microbial effects seem unlikely because the pH was maintained at pH 10, and oxidative problems should have been eliminated, or made similar across treatments, by the presence of the antioxidant Na_2SO_3 in the grinding buffer.

When the results of different experiments are compared, the same general behaviors are apparent. However, the absolute storage time to an effect may differ enormously. Thus, in homogenates made from defoliated shrub and with an initial concentration of <4 mg/ml latex, degradation began after 3 weeks in one experiment (Fig. 1) but not until after 32 weeks in another (Fig. 4B). Also, the behavior of homogenate in which the pH was unregulated may vary. In the pH experiment (Fig. 2), substantial declines in latex levels did not occur until after 26 weeks. In contrast, a 1326-l batch of poorly pH-controlled homogenate made from shrub still with some leaves, became rancid and lost 75% of its latex after only three days (unpublished data). However, the homogenate in Fig. 2 was adjusted to pH 10 when it was first made. Although the pH then quite rapidly fell, the initial pH 10 treatment appeared to have had a substantial and lasting inhibitory effect. The 1326-l batch was probably never much above pH 7 and microbial and enzyme activities were not destroyed or irreversibly inhibited.

We have been unable to pinpoint the cause of all differences observed nor why latex is more unstable at low concentration. However, from a practical viewpoint,

unrefrigerated homogenates should be stored only if the initial latex concentration is at least 4 mg/ml regardless of the branch size or post-harvest storage history of the shrub. In an exception to this statement, in our laboratory tests, latex levels below 4 mg/ml could be maintained for many weeks in homogenates made from leafy shrub instead of defoliated shrub (Fig. 4A). This improvement in latex stability may result from natural antioxidants, such as carotenoids, released into the homogenate from the leaves during grinding. Nonetheless, in a commercial bioprocessing plant, 8 weeks should be ample time to repair a mechanical failure in the processing operation, and longer storage times are unlikely to be needed.

No determinations of the molecular characteristics of the latex were made during the storage experiments reported in Figs. 1–3. However, the physical properties of the suspended rubber particles in the latex did change noticeably after the homogenate had been stored for more than 17 weeks at pH 10, and much earlier in the pH-unregulated homogenate; the coagulated rubber produced by the latex quantification method (Cornish et al., 1999) became softer and more difficult to harvest from the tubes, making the results less reproducible. The physical changes likely reflect low molecular weight rubber and the presence of degradation products that plasticize the rubber.

4.2. Latex quality

Plant polymers are generally not monodispersed and various sizes exist in vivo during biosynthesis and sequestration. In processed material, polymer size is affected by degradation during harvesting and sample preparation. Since the molecular weight of the latex rubber is closely correlated with rubber quality (Swanson et al., 1979), molecular characteristics were used in this study as a measure of latex quality equating high molecular weight (>1,000,000 g/mol) to good quality. Molecular weights (1,000,000–1,600,000 g/mol) of latex in initial samples indicate that latex quality would be good when prepared rapidly from fresh guayule.

Storage of guayule, particularly at 24 °C, changed molecular structure of the latex molecules (Figs. 5 and 6) reducing quality. Refrigeration (4 °C) offered some protection against decreases in molecular weight and dynamic radius (Figs. 5 and 6). Extracted latex consisted of molecules varying in molecular weights as indicated by the polydispersity index (Fig. 7). It was apparent that rubber polymer molecular weight can significantly decline even when no change in latex yield is detectable (cf. Figs. 4A and 5A) and that refrigeration, although it protects yield (Fig. 4), does not prevent molecular weight decreases (Fig. 5). However, under refrigeration rubber molecular weight did not decline below 500,000 g/mol (Fig. 5) during the 66 weeks of the experiment. The difference in minimum molecular weights of latex rubber in homogenates stored at 4 or 24 °C suggests that two processes, of which only one was inhibited by cold, are responsible for degradation of the latex. Heterogeneity may exist in the guayule rubber polymers and be responsible for the resistance to degradation below 500,000 g/mol seen in refrigerated samples (Figs. 5 and 6). The degradation of the polymers observed at 24 °C, but not at 4 °C suggests an enzymatic degradative process. In contrast, the degradation of the polymer from its original size down to near 500,000 g/mol that occurs at 4 °C is probably a chemical process, such as oxidation, but it is not understood why this does not continue throughout the storage period unless the oxidant is being consumed. The presence of distinct degradative processes also is indicated by the initial reduction in molecular weight (Fig. 5) seen at both temperatures only being matched by a change in conformation to the more tightly packed configuration at 24 °C (Fig. 8). However, because the polydispersity indices remain near 1 (Fig. 7) throughout the large decline in quantity (Fig. 4) and molecular weight (Fig. 5) that occurs during the 66-week homogenate storage period, the rubber molecules probably are not being degraded by internal attacks, which would generate different polymer sizes and a large increase in polydispersity. Instead, we suggest that the polymers are predominately degraded from their free ends in pieces too small to be detected by SEC-MALLS analysis. The specific mechanism for this degradation is not yet known. Also, the rubber particles in the homogenate undergo the degradative processes simultaneously. If latex included a mixture of resistant and susceptible particles degrading at different times, a mixture of different polymer sizes would result, which would in turn generate high polydispersity values. This is, in fact, what occurs in shrub that has been stored

post-harvest at 40 °C, where polydispersity values near 4 are obtained for the extracted latex polymers (Cornish et al., 2002a, 2002b).

The stability of latex quality in the homogenates is not much affected by defoliation. The only exception appears to be a decrease in stability caused by leaf tissue in the second grind homogenate stored at 24 °C (Fig. 5; Table 1), but this may largely be a dilution effect. This is in contrast to the stability of latex yield, in which the presence of leaf material appeared to enhance stability (Figs. 1 and 4).

5. Conclusions

Guayule rubber molecular weights of over 1,000,000 g/mol in freshly extracted latex indicate that latex quality is initially excellent. Storage of homogenate without refrigeration reduces molecular weight and changes the molecular structure of the rubber polymers reducing quality. However, homogenate appears to provide a stable environment for latex for 13–16 weeks, without refrigeration, provided that the pH is basic and the concentration of rubber particles is at least 5 mg/ml. This is in contrast with the extractable latex content of harvested branches, which is prone to coagulation and degradation in situ unless the branches are stored hydrated and refrigerated (Cornish et al., 2000). Latex stability in homogenates was minimally affected by shrub storage post-harvest prior to homogenization nor by the degree of shrub foliation. Therefore, if delays in a latex extraction and purification process occur post-harvest, losses should be minimal provided that the shrub is homogenized.

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